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(54) Title: HUMANISED ANTIBODIES HAVING MODIFIED ALLOTYPIC DETERMINANTS

(57) Abstract

The invention relates to molecules which have an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain. The constant regions are of a particular isotype and have one or more allotypic determinants. The amino acid sequence is substantially homologous to the amino acid sequence of the constant region. However, it has been altered so that it is without at least one of said allotypic determinants by making its sequence the site for an allotypic determinant identical to the amino acid sequence from the corresponding position in another equivalent immunoglobulin constant region of a different isotype. The invention provides synthetic immunoglobulins with reduced allotypic differences as compared to a given wild-type immunoglobulin.

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Humanised antibodies having modified allotypic determinants

The present invention relates to binding molecules. In particular, it relates to recombinantly produced antibodies.

Owing to their high specificity for a given antigen, antibodies and particularly monoclonal antibodies (Kohler, G. and Milstein C., 1975 Nature 256:495) represented a significant technical break-through with important consequences scientifically, commercially and therapeutically.

Monoclonal antibodies are made by establishing an immortal cell line which is derived from a single immunoglobulin producing cell secreting one form of a biologically functional antibody molecule with a particular specificity.

Owing to their specificity, the therapeutic applications of monoclonal antibodies hold great promise for the treatment of a wide range of diseases (Clinical Applications of Monoclonal Antibodies, edited by E. S. Lennox. British Medical Bulletin 1984, publishers Churchill Livingstone). Antibodies are generally raised in animals, particularly rodents, and therefore the immunoglobulins produced bear characteristic features specific to that species. The repeated administration of these foreign rodent proteins for therapeutic purposes to

human patients can lead to harmful hypersensitivity reactions. In the main therefore, these rodent-derived monoclonal antibodies have limited therapeutic use. A further problem with these rodent derived antibodies, is that they are relatively ineffective at the depletion of cells in vivo, although the rat IgG2b antibody CAMPATH-1G is an exception to this rule.

Thus, there is a need for therapeutic antibodies which have characteristic features specific to human proteins. Unfortunately, immortal human antibody-producing cell lines are very difficult to establish and they give low yields of antibody (approximately 1 µg/ml). In contrast, equivalent rodent cell lines yield high amounts of antibody (approximately 100 µg/ml). Furthermore, where one wants to produce a human antibody with a particular specificity it is not practically or ethically feasible to immunise humans with an immunogen bearing the epitope of interest.

In part, this problem has been overcome in recent years by using the techniques of recombinant DNA technology to 'humanise' non-human antibodies. Structurally, the simplest antibody (IgG) comprises four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulphide bonds (see figure 1). The light chains are of two types, either kappa or lambda. Each of the H and L chains has a region

of low sequence variability, the constant region (C) giving rise to allotypes and a region of high sequence variability, the variable region (V) giving rise to idiotypes. The antibody has a tail region (the Fc region) which comprises the C regions of the two H chains. The antibody also has two arms (the Fab region) each of which has a V_L and a V_H region associated with each other. It is this pair of V regions (V_L and V_H) that differ from one antibody to another, and which together are responsible for recognising the antigen. In even more detail, each V region is made up from three complementarity determining regions (CDR) separated by four framework regions (FR). The CDRs are the most variable part of the variable regions, and they perform the critical antigen binding function. The CDR regions are derived from many potential germ line sequences via a complex process involving recombination, mutation and selection. It has been shown that the function of binding antigens can be performed by fragments of a whole antibody. Binding fragments are the Fv fragment which comprises the V_L and V_H of a single heavy chain variable domain (V_H).

In creating "humanised" immunoglobulins, the Fc tail of a non-human antibody is exchanged for that of a human antibody. For a more complete humanisation, the FRs of the non-human antibody are exchanged for human FRs. This

process is carried out at the DNA level using recombinant techniques. However, these humanised immunoglobulins do not solve all the problems, because an immune response may still be mounted against the treatment antibody even when a patient is treated with a human antibody, as it may show certain sequence differences in the V (ie idiotypic differences) and C (ie allotypic differences) regions when compared with the patients own equivalent antibodies. This is a particular problem where the patient's immune system has already seen, and therefore been primed against, antibodies having these sequence differences (eg a patient may have received a prior blood transfusion which contained allotypically different immunoglobulins). A model system of injecting "mouseised human antibodies" into mice indicated that the allotype matching could critically affect the anti-idiotype response (Bruggemann M., Winter G., Waldmann H., Neuberger M.S., (1989) J. Exp. Med. 170, 2153-2157).

The present applicants have realised that one way around this problem is to eliminate the allotypic variation from the constant region.

There are a range of different immunoglobulins IgG, IgM, IgA, IgD, IgE, known as isotypes, of which IgG is most commonly used therapeutically. It exists as isotypic sub-classes IgG1, IgG2, IgG3 and IgG4.

There are 24 recognised allotypes of human

immunoglobulin distributed between the different isotypes as follows:

IgG1	x	4
IgG2	x	1
IgG3	x	13
IgA2	x	2
IgE	x	1
Kappa	x	3

The allotypes represent alternative amino acid substitutions found at discrete sites in the protein sequence. These different allotypic determinants are found in different combinations within given allelic forms of genes, but not all possible combinations which theoretically might exist are in practice observed.

For example, the four different allotypes of IgG1 can be seen (ie distinguished) by the immune system. These are G1m 1, 2, 3 and 17. Alternatively, combinations thereof, such as G1m (1, 17), can also be distinguished. The four different single allotypes are depicted in figure 2.

Antisera can be raised in other non-human species which can see the alternative isoallotypes provided that the antibody is purified away from the other human isotypes. Such isoallotypes for which such an antisera exists have been called non-allotypes and given the designation for example, nG1m(1) which is the isoallotype

of Glm(1). Thus, although a human isoallotype should not be immunogenic in humans, it can still potentially be recognized in a different species.

Of the above mentioned different allotypes of IgG1, three common allelic forms of human IgG1 occur with different frequencies within different racial groups, namely Glm (3), Glm (1, 17), and Glm (1, 2, 17) based upon their reactivities with human antisera directed against the determinants Glm 1, 2, 3 and 17. At some point in the future, it is likely that a patient with an existing anti-allotype response to one or more of these determinants will need treatment with a humanised antibody. The obvious solution and one which has been proposed in a letter to the Journal Nature (Mage, R.G., Nature (1988) 333, 807-808), is to make all the different allelic forms of an antibody and to allotype match each patient for therapy. The present applicants have realised that commercially this is not a good proposal because of increased production costs and the need to process several reagents in parallel through the regulatory requirements. Additionally, each patient would have to be tested for the response to different allotypes.

Thus, the present applicants propose eliminating the allotypes altogether from each therapeutic antibody. The sequence of the human allotype of IgG1 Glm (1, 2, 17) is

shown aligned with sequences for the other human IgG, isotype sub-classes in figure 4 (a, b, c and d). It can be seen that the four isotypes are extremely homologous for the domains CH1, CH2 and CH3, and that the major isotypic differences are in the hinge region which varies in both, length and sequence between isotypes. The allotypic residues of IgG1 G1m (1, 2, 17) have been marked in figure 4. However, for the purposes of clarity the sequences around the allotypic sites G1m (1) (2) and (17) are shown below for each isotype.

Site (1)

355	356	357	358	
Arg	Asp or Glu	Glu	Leu or Met	IgG1
Arg	Glu	Glu	Met	IgG2
Arg	Glu	Glu	Met	IgG3
Gln	Glu	Glu	Met	IgG4

Thus, at site (1), IgG1 may exist as several allotypes depending on whether aspartic acid or glutamic acid at position 356, or leucine or methionine at position 358 are present.

Site 2

430	431	432	
Glu	Gly or Ala	Leu	IgG1
Glu	Ala	Leu	IgG2
Glu	Ala	Leu	IgG3
Glu	Ala	Leu	IgG4

Thus, at site (2), IgG1 may exist as either of two allotypes depending on whether glycine or alanine is present at position 431.

Site (17)/(3)

Sites (3) and (17) are alternative substitutions at the same site.

213	214	215	
Lys	Lys or Arg	Val	IgG1
Lys	Thr	Val	IgG2
Lys	Arg	Val	IgG3
Lys	Arg	Val	IgG4

Thus, at site (17)/(3), IgG1 may exist as either of two allotypes depending on whether lysine or arginine is present. The allotypes (17) and (3) cannot co-exist as they represent alternative substitutions at the same position.

The alternative alleles of G1m (1) and (2) do not provoke a human allotype response because of the homology of these alleles with the other IgG sub-classes in this region. These alleles are therefore called isoallotypes because they are only recognisable by xenoantisera (antisera from a different species) and only when the isotype is purified away from the other sub-classes.

Therefore, the present applicants propose the creation of a new IgG1 allele by site-directed mutagenesis of the gene, for example, an existing

CAMPATH-1H monoclonal antibody gene described below, so that the new allele consists entirely of isoallotypic determinants. The preparation of IgG1 mutants according to the teaching provided by the present applicants is shown schematically in figure 3.

For G1m (1) and G1m (2), the changes comprise simple substitution by the alternative isoallotypic residues. However, in the case of G1m (17) the conversion of lysine to arginine would in some cases merely change the allotype to an allotype that is recognised by certain individuals as a G1m (3) allotype despite the fact that this residue is homologous with IgG3 and IgG4. This apparent contradiction is thought to be because this arginine is seen in a tertiary epitope in the context of the other IgG1 specific residues in close proximity in the CH1 domain or hinge region. This indicates that in addition to changing lysine, other residues in CH1 or the hinge will need to be changed in order to create a new isoallotype.

Although the above and ensuing description is specifically directed to IgG1 and in particular, the CAMPATH-1H monoclonal antibody, the same approach can be used to create isoallotypes of the other human isotypes such as IgG2, IgG3 and kappa.

Thus, the present invention provides a first binding molecule derivable from a second binding molecule;

which second binding molecule is an immunoglobulin, or a derivative, structural or functional analogue thereof, a member of a family of homologous molecules, and has one or more sites which are structurally distinctive from equivalent sites in the other family members;

wherein said first binding molecule is more closely homologous to the other family members than to said second binding molecule, at at least one of said one or more sites.

The first binding molecule may also be an immunoglobulin or a derivative, structural or functional analogue thereof. The one or more sites which are structurally distinctive from the equivalent sites in the other family members may be in the constant region giving rise to an allotypic difference. The first binding molecule may comprise entirely isoallotypic determinants.

The second binding molecule may be selected from the group consisting of IgG1, IgG2, IgG3, IgA2, IgE, kappa light chains or derivatives, structural or functional analogues thereof. Where the second binding molecule is IgG1, the allotypic differences may be present at one or more of sites (1) (2) (3) or (17) as described herein. Where the second binding molecule is IgG2, the allotypic difference may be present at site (23). Where the second binding molecule is IgG3, the allotypic differences may

be present at one or more of the sites (11) (5) (13) (14) (10) (6) (24) (21) (15) (16) (26) or (27). Where the second binding molecule is IgA2, the allotypic differences may be present at one or more of the sites (1) and (2). Where the second binding molecule is kappa light chain, the allotypic differences may be present at one or more of the sites (1) (2) or (3). The sites referred to above are well documented in the literature (see e.g. Eur. J. Immunol. 1976.6:599-601. Review of the notation for the allotypic and related marks of human immunoglobulins).

The present invention also provides pharmaceutical preparations comprising a first binding molecule as defined above or described herein together with one or more excipients. The pharmaceutical preparation may comprise a cocktail of said first binding molecules.

Also provided by the present invention are methods for making a first binding molecule as defined above or described herein.

These methods comprise the steps of: a) identifying in said second binding molecule, one or more sites which are structurally distinctive from the equivalent sites in the other family members; b) making said first binding molecule whereby it is more closely homologous to the other family members than to said second binding molecule at at least one of said one or more sites.

The first binding molecule may be made by providing a gene sequence encoding the second binding molecule and altering those parts of the gene sequence encoding said one or more sites. The gene sequence may be altered by site directed mutagenesis using oligonucleotide primers. The altered gene sequence may be incorporated into a cloning vector or expression vector. The expression vector may be used to transform a cell. The cell may be induced to express the altered gene sequence.

The present invention therefore provides cloning vectors and expression vectors incorporating the altered gene sequence. Also provided are cells transformed by expression vectors defined above. Also provided are cell cultures and products of cell cultures containing the first binding molecules. Also provided are recombinantly produced said first binding molecules.

Thus the present invention provides a molecule which comprises an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain which constant regions are of a particular isotype and have one or more allotypic determinants

wherein said amino acid sequence is substantially homologous to the amino acid sequence of said constant region, but has been altered so that it is without at least one of said allotypic determinants, by making the amino acid residues at the site of an allotypic

determinant identical to the amino acid residues from the corresponding position in another equivalent immunoglobulin constant region of a different isotype.

The molecule may comprise an amino acid sequence derivable from part or all of a human immunoglobulin constant region.

The molecule may also comprise one or more polypeptides together with said amino acid sequence.

The polypeptide may comprise a functional biological domain. The domain may be such that it mediates any biological function. The functional biological domain may comprise a binding domain. The binding domain will have an ability to interact with another polypeptide. The interaction may be non-specific or specific.

The polypeptide, biological domain, binding domain and immunoglobulin-like binding domain may derive from the same source or a different source to the constant region.

The constant region may be from an immunoglobulin of the isotype IgG. The isotype subclass may be IgG1 and the molecule may no longer have one or more of the allotypic determinants 1,2,3 and 17. The isotype subclass may be IgG2 and the molecule may no longer have the allotypic determinant 23. The isotype subclass may be IgG3 and the molecule may no longer have one or more of the allotypic determinants 11,5,13,14,10,6,24,21,15,

16,26 and 27.

The constant region may be from an immunoglobulin of the isotype IgA2 and the molecule may no longer have either or both of the allotypic determinants 1 and 2.

The present invention also provides a pharmaceutical preparation which comprises a molecule as defined.

The present invention also provides a reagent which comprises a molecule as defined.

The present invention also provides a nucleotide sequence encoding a molecule as defined.

The present invention also provides cloning and expression vectors comprising a nucleotide sequence as delivered above.

The present invention also provides host cells comprising a cloning or expression vector as defined above.

The present invention also provides a method of preparing a molecule as defined above which comprises the steps of:

(a) identifying a constant region of an immunoglobulin heavy chain;

(b) comparing the identified constant region with constant regions from immunoglobulin heavy chains of the same isotype to locate allotypic determinants in the identified constant region;

(c) obtaining the coding sequence for the identified

constant region having allotypic determinants;

(d) altering the coding sequence so that it codess for a molecule without at least one of said allotypic determinants and by making the amino acid residues at the site for an allotypic determinant identical to the amino acid residues from the corresponding position in an equivalent immunoglobulin constant region of an isotype different to that of said identified constant region;

(e) using said altered coding sequence in an expression system to produce a said molecule.

The present invention also provides a method of treating a patient which comprises administering a pharmaceutical preparation as defined above.

Of course, there are a number of different strategies which could be used in order to make the molecules with fewer allotypic determinants.

Genes encoding therapeutically useful antibodies are generally available in one of several different forms. They may be available as a cloned variable region DNA sequence with restriction sites at each end, suitable for recloning along with a chosen cloned constant region DNA sequence into a suitable expression vector. This is the strategy described herein for the constructs TF57-19, MTF121 and MTF123. Alternatively, they may be available as complete immunoglobulin DNA sequences including the V and C regions together, e.g. a cDNA clone of a complete

humanised or human antibody.

Whatever the form in which the cloned immunoglobulion gene is obtained, the next step is to predict the amino acid sequence of the constant region from the DNA sequence. The DNA sequence can be obtained using a variety of strategies familiar to molecular biologists. The predicted amino acid sequence would then be checked for the amino acids known to vary as allotypes. Any isoallotypes present within the sequence can be left unaltered. Any allotypes present can be mutated.

The next step, is to decide what amino acid sequence to mutate the allotype to, in order to imitate an isoallotype. This is done by lining up the sequence with the corresponding region of the other immunoglobulin isotypes. For all known allotypes, it has been found that one or more of the other isotypes have invariant sequences for the homologous region. One of these sequences can then be chosen to form the basis for the changes to be made in the allotype in question. Having predicted the new amino acid sequence for the constant region, it is necessary to alter the existing DNA clone or to create a new DNA clone which will encode this sequence. Again there are several strategies available to molecular biologists in order to achieve this. In the case of the example CAMPATH-1H constructs described

herein, the gamma-1 constant region was cloned in an M13TG131 single stranded phage vector. Mutagenic oligonucleotides were synthesised which were largely homologous to the single strand, but which contained base changes necessary to alter the codons for the critical amino acids. The mutagenesis was carried out using a commercial kit from Amersham International, High Wycombe, Bucks. Alternatively it would be possible to synthesise a complete artificial gene which encodes the predicted sequence.

Once mutated or newly synthesised, the gene is ready for expression. There are many different expression vectors available. Some of these are more suitable for expression in restricted cell types. Again it is within the standard technical expertise of one skilled in this field to choose and adapt expression vectors for this purpose. In the case of the CAMPATH-1H constructs described herein, modifications of the pSVgpt and pSVneo vectors have been used. These vectors have convenient cloning sites for the immunoglobulin variable and constant region, encoding DNA fragments adjacent to suitable promoter and enhancer sequences to allow expression in lymphoid cells. The vector allows the easy independent replacement of variable or constant region encoding DNA fragments. Thus, any suitable variable region can be subcloned into the vector, to give rise to

a new specificity, or the variable region can be kept and the constant region changed to give rise to a new isotype or allotype. Alternative vector systems are readily available.

Having removed allotypes from heavy chain constant regions by mutating them all to isoallotypes, it may still be desirable to consider the light chain effect in stimulating an immune response.

The most common kappa light chain allotype is Km(3) in the general population. Therefore it may be sufficient to utilise this common kappa light chain allotype, as relatively few members of the population would see it as foreign.

Alternatively there are no lambda light chain allotypes. Therefore they could be used in combination with the de-allotyped molecules derivable from heavy chain constant regions.

Where one utilises the kappa light chain, the allotype Km(1,2) could first be mutated to the allotype Km(1). The light chain allotype Km(1) is often only weakly recognized in combination with certain heavy chain classes and subclasses, and so may not cause a major problem in therapeutic use.

In order that the present invention is more fully understood embodiments will now be described in more detail, by way of example only, and not by way of

limitation. Reference will be made (and has already been made in the text above) to the following figures in which:

figure 1 illustrates the structure of an IgG antibody;

figure 2 shows the allotypes for the IgG1 antibody CAMPATH-1H;

figure 3 shows schematically the preparation of IgG1 mutants;

figure 4 shows the IgG1 G1m (1,2,17) allotype sequence aligned to single allotypic examples of IgG2, 3 and 4 (none of these other subclasses have allotypic residues which cover the same residues as for the IgG1 allotypes);

figure 5 shows the M13TG131 cloning vector containing the human gamma-1 constant region, showing cloning sites and modified polylinker;

figure 6 shows the original Hu4vH HuG1 pSVgpt expression vector and its modified version;

figure 7 shows the result of an ELISA assay testing different dilutions of the antibodies of mutants 1, 2 and wild type CAMPATH-1H for IgG1 specificity;

figure 8 shows the result of an autologous complement mediated lysis test using human peripheral blood lymphocytes; and

figure 9 illustrates an antibody-dependent cell-

mediated cytotoxicity assay (ADCC) using CD3 activated interleukin-2 expanded human blastocytes cell effectors (E) and targets (T).

The starting antibody used for site-directed mutagenesis was CAMPATH-1H, a monoclonal antibody with a kappa light chain containing the human constant region sequence for IgG1 which carries the G1m (1, 17) allelic determinants. The whole IgG1 encoding region exists as approximately 2.3 kb HindIII-SphI restriction fragment cloned in an M13 vector. The M13TG131 cloning vector containing the human gamma-1 constant region showing cloning sites and modified polylinker is shown in figure 5.

The IgG1 encoding region is entered in the EMBL Sequence Database under the code number HS1GCC4. The accession number is AC J00228 (the printout from the database is provided herein as Appendix 1). This sequence is for the G1m (1, 17) allotype. It covers 2009 bases from the 5' HindIII site (A)AGCTT including all of the coding region. It does not however, include some of the 3' non-coding region up to the SphI site. The sequence provided by the EMBL Database is that of the upper strand of DNA. The CH1 domain starts at nucleotide 210 and ends at nucleotide 503. The mutagenic oligonucleotides M01 and M04 hybridise to nucleotides 486 to 510. The hinge region starts at nucleotide 892 and

ends at nucleotide 936. The CH2 domain starts at nucleotide 1481 and ends at nucleotide 1803. The mutagenic oligonucleotide MO2 hybridises to nucleotides 1515 to 1543. The essential signal for the poly A tail is provided by nucleotides 1902 to 1908.

In M13TG131, the IgG1 coding region exists as a 2260 nucleotide fragment, of which the final 251 nucleotides are non-coding and therefore, inessential. Therefore, an embodiment of the invention could be carried out using the sequence information provided by the EMBL Sequence Database. It should be noted however, that the Sph1 restriction site referred to above, is present in the 3' end inessential non-coding region. Therefore, if the sequence data as provided by the EMBL database were being used, alternative restriction sites would have to be utilised.

Using site-directed mutagenesis, (carried out using protocols and reagents as supplied in kit form, Amersham code RPN. 1523, Amersham International Plc, Amersham, UK) the sequence corresponding to the G1m (1) allele was converted to the corresponding sequence found in the other sub-classes for IgG (Asp Glu Leu to Glu Glu Met at positions 356-358 in the CH3 domain).

The mutagenic oligonucleotides used were:

a) MO1 (to convert G1m (17) to G1m (3))

5' CTC TCA CCA ACT CTC TTG TCC ACC T 3';

b) MO2 (to convert Glm (1) to its isoallotype nGlm (1))
5' GGT TCT TGG TCA TCT CCT CCC GGG ATG GG 3'; and

c) MO4 (to eliminate Glm(3) by changing Lys to Thr in the CH1 region)
5' CTC TCA CCA ACA GTC TTG TCC ACC T 3'.

The oligonucleotides were synthesised and then purified using an automated synthesizer and oligo purification columns supplied by Applied Biosystems (Applied Biosystems, 850 Lincoln Drive, Foster City, California, 94404 USA) following the manufacturers recommended protocols. Mutations were checked by Sanger Dideoxy sequencing (Sanger, F.S., Nicklen, S., and Coulson, A.R., (1977) Proc. Natl. Acad. Sci., USA, 74, 5463) using standard protocols and kits. As this newly formed allotype sequence is found in all humans, there should be no immunological response to this alternative form of Glm (1). Additionally and similarly, the lysine residue responsible for the Glm (17) allotypic determinant was converted to an arginine residue corresponding to the Glm allele (Lys 214-Arg; mutant 1).

The gene for this new constant region of mutant 1 carrying these three changes has been sequenced, incorporated into an expression vector containing the CAMPATH-1H V-region and expressed together with the CAMPATH-1H light chain which had been introduced by co-transfection.

A further mutant has been made by replacing the critical arginine residue associated with Glm (3) of mutant 1 with a threonine residue, to produce a heavy chain which is the equivalent of IgG2 and which should fail to react with both anti-Glm (17) and anti-Glm (3) antisera (mutant 2).

Mutant 2 has also been sequenced, re-cloned in an expression vector containing the CAMPATH-1H light chain.

The supernatants of growing cultures containing either of the two mutants were subsequently assayed for the expression of a human IgG1 kappa product.

The mutations were introduced with the oligonucleotides listed above. The modified Hu4vHG1pSVgpt vector shown in figure 6 was used to simplify the subcloning of all the new mutants into the expression vector, owing to the possibility of use of two different "sticky ends" Bam HI and NotI. The expression vectors and V_H region sequences and expression, along with the light chains, in YO rat plasmacytoma cells are all as described in Riechmann L., Clark, M.R. Waldman H., Winter G. (1988) Nature 332, 323-327.

From the positive cultures, the producers of the largest amount of the IgG1 product were selected to obtain purified antibody for biological assays to determine their allotypes and biological effector functions.

Example 1

An Enzyme-linked Immuno Sorbent Assay (ELISA) was performed to verify that an IgG1 type antibody was produced by the mutants. This was tested with microtiter plates coated with anti-CAMPATH-idiotype antibody (YID 13.9). Wild type CAMPATH-1H antibody served as control. The bound antibody was detected with biotin-labelled anti-human kappa reagents or anti-human IgG reagent (monoclonals NH3/41 and NH3/130 respectively although other suitable reagents are commonly available) and subsequent development with streptavidin horseradish peroxidase. Figure 7 illustrates the results obtained for:

TF 57-19 ("wild type" CAMPATH-1H antibody, O)
MTF 121 (mutant 1,▲)
MTF 123 (mutant 2,■)

and the wild type CAMPATH-1H (▼) in a known amount as standard. The concentrations had been estimated, and the starting dilutions adjusted to 50 μg/ml in PBS/10 mg/ml BSA. The starting dilution was used to prepare 8 two-fold dilutions.

The slope of the graph shows clearly that the CAMPATH-idiotype antibodies recognises mutants 1 and 2 to an extent equivalent to that of the wild type CAMPATH-1H, and that all three antibodies tested are present in similar concentrations as the standard.

Example 2

The mutants' capability of autologous complement mediated lysis of human peripheral blood lymphocytes was tested.

Human peripheral blood mononuclear cells from a healthy donor were isolated from 60 ml defibrinated blood on a Lymphoprep* gradient (Nyeggard & Co., AS, Oslo, Norway). The cell pellet was washed in IMDM (Iscove's Modification of Dulbecco's Medium, Flow Laboratories, Scotland), and the cells were labelled with ^{51}Cr. The starting dilution of antibodies used in the test was 50 µg/ml in PBS, 10 µg/ml BSA (dilution 1). Dilution 1 was further diluted 8 times two-fold to a final dilution of 1/128. Wild type antibody diluted in the same manner was used as a control.

The result is illustrated in figure 8. As can be seen, both antibody mutants show a very similar result in lysing the blood mononuclear cells as the wild type. The efficiency of the mutants is almost identical.

Example 3

Experiments were conducted to investigate whether or not, the mutant antibodies were capable of antibody-dependent cell-mediated cytotoxicity (ADCC) using CD3 activated interleukin-2 expanded human blastocytes as effectors (E) and targets (T). Cells were generated and used as both effectors and targets essentially as

described in Riechmann L., Clark M.R., Waldmann H., Winter G., 1988, Nature 322, 323-327.

Preparation of Target Cells (T)

5 ml of blastocytes (3×10^6 cells) were labelled with ^{51}Cr for 1 h. After 1 h the cells were washed and transferred in 6 equal aliquots in 100 µl IMDM 1% BSA, to 6 x 10 ml tubes containing 100 µl of the antibodies of mutants 1 and 2, and the control. The tubes were incubated for 1.5 h at room temperature. The cells were then washed with 10 ml IMDM 1% BSA and resuspended in 1.5 ml IMDM 1% BSA.

Preparation of Effector Cells (E)

Unlabelled blastocytes (2×10^6) were diluted 100:1 and 30:1 in IMDM 1% BSA medium. The ratios 100:1 and 30:1 refer to the final absolute ratios of effectors to ^{51}Cr labelled targets in the assay. Assays were performed in microtitre plates with a total volume of 200 µl per assay well. Thus 100 µl of targets at a concentration of 2×10^4 were put in each well ie 2×10^3 total cells. For E:T of 100:1, 100 µl of effectors at 2×10^6 were plated per well ie 2×10^5. For E:T of 30:1 100 µl of effectors at 6×10^5 were put into each well ie 6×10^4 total cells.

The efficiency percentage of specific ^{51}Cr release was calculated as follows:

% specific ^{51}Cr release =

$$\frac{\text{(test release cpm - spontaneous (cpm) x 100}}{\text{(total cpm - spontaneous cpm)}}$$

cpm = radioactive counts per minute as measured on a counter.

The result is shown in figure 9. The figure shows that all of the antibodies tested released chromium. Wild type TF 57-19 and mutant 2 (MTF 123) released at about equal levels, whereas mutant 1 (MTF 121) shows a slightly higher release.

These results clearly show that the mutants have biological activity comparable to the wild type CAMPATH-1H antibody.

Example 4

The antibodies were tested in an assay specific for their Glm (3) allotypes reactivity using a monoclonal reagent from Oxoid (WHO/IVISS recognised agent, Study Code No HP 6027). These tests were performed in replicates of two.

Microtiter plates were coated with the anti-CAMPATH idiotype YID 13.9.4 antibody captive, and divided into three arrays of 4 x 4 wells. Into each of the three arrays, 4 x 5 fold dilutions of the antibody TF 57-19, MTF 121 and MTF 123 (50 µg/ml) in PBS 1% BSA and a control solution of PBS/BSA each were added.

After an incubation of 45 minutes at room temperature, the antibody solution was removed, and

(i) to the first array was added a 1:500 dilution of biotin-labelled anti-G1m (3);

(ii) to the second array was added a 1:100 dilution of biotin-labelled antibody (NH3/41) specific for the kappa light chain; and

(iii) to the third array was added a 1:1000 dilution of biotin-labelled antibody (NH3/130) specific for human IgG1.

The microtiter plate was developed with streptavidin horseradish peroxidase.

The result is illustrated in Table 1. The numbers in the results represent the optical density (O.D) as measured in an ELISA plate reader multiplied by 100 ie 12 represents an O.D of 0.12 and 70 an O.D of 0.70.

The result clearly shows, that samples 1-3 all react with the antibodies specific for IgG1 (see also Example 1 above) and the kappa light chains. The control is negative. However, in the assay for G1m (3) specificity, only MTF 121 (mutant 1) shows reactivity, whereas the wild type TF 57-19, MTF 123 (mutant 2) and the PBS/BSA control did not show any response.

This result illustrates clearly that the elimination of sites recognised in the allotype response by site-directed mutagenis of these sites can overcome the problems of allotypic immuno-reactions. Although the examples refer to the mutagenesis of IgG1 only, it will

be clear to the person skilled in the art that other immunoglobulin isotypes can be similarly modified.

Example 5

The antibodies were tested in a conventional allotyping experiment using inhibition of red cell agglutination. The experiment was carried out using reagents supplied by the Central Laboratory of the Netherlands Red Cross, Blood Transfusion Service (PO Box 9190, 1006 AD Amsterdam, Netherlands).

Human blood group O Rhesus D red cells were washed and then aliquots separately labelled as described below with one of the following three relevant anti-RhD human sera having antibodies of known allotype.

(1) anti-D Glm(az) = Glm (1,17)

(2) anti-D Glm(x) = Glm (2)

(3) anti-D Glm(f) = Glm (3)

Coating of Red Cells with Anti-Rh Antibodies

One volume of packed washed red blood cells were incubated with 4 volumes anti-Rh serum and 4 volumes (phosphate) buffered saline (PBS) at 37°C during 60 minutes. Every 15 minutes the cells were mixed by shaking.

After incubation the coated cells were washed four times with PBS and stored at 4°C in preservation fluid (although coated red blood cells can be stored at 4°C in PBS for one week).

These coated red blood cells were then agglutinated with four antisera to the IgG1 allotypes as follows using the recommended dilution for each antiserum.

(1) anti-G1m(z) = anti-G1m(17) 1 in 30 dilution

(2) anti-G1m(a) = anti-G1m(1) 1 in 30 dilution

(3) anti-G1m(x) = anti-G1m(2) 1 in 20 dilution

(4) anti-G1m(f) = anti-G1m(3) 1 in 30 dilution

The wild-type CAMPATH-1H TF57-19 or the different CAMPATH-1H constructs (MTF 121, MTF 123) with the altered gamma-1 constant regions were then tested for their abilities to inhibit the agglutination of the red cells by the above antisera. The inhibiting antibodies were tried at concentrations of 0.5mg/ml, 0.25mg/ml and 0.125mg/ml in phosphate buffered saline containing 5% foetal bovine serum. Control sera containing IgG1 of allotype G1m(zax) or G1m(f) [G1m(1,2,17) or G1m(3)] were also included in the experiment and were used at dilutions of 1 in 10,20 and 40. Where it occurred the inhibition was most easily seen for the CAMPATH-1H antibodies at the 0.5mg/ml concentration and it was much weaker for 0.25mg/ml and no inhibition was seen at 0.125mg/ml. The control sera inhibited at all three dilutions tested. The results for the highest concentration are shown below.

Allotype CAMPATH-1H constructs Control sera

	TF57-19	MTF121	MTF123	Glm(1,2,17)	Glm(3)
Glm(1)	+	-	-	+	-
Glm(2)	-	-	-	+	-
Glm(3)	-	+	-	-	+
Glm(17)	+	-	-	+	-

The results are therefore consistent with the original wild type CAMPATH-1H antibody TF57-19 having allotype Glm(1,17). The new mutant MTF121 type as allotype Glm(3) whilst the mutant MTF123 fails to allotype for any of the IgG1 allotype markers Glm(1,2,3,17) i.e. it appears not to have an IgG1 allotype.

The skilled man will be able to use the binding molecules hereby provided to make pharmaceuticals according to standard techniques. Similarly the pharmaceuticals can be used in accordance with standard practices.

Table 1

Type Specific Antibody	anti G1m(3)				anti kappa (NH3/41)				anti IgG 1 (NH3/130)			
Sample (dilution)	10	2	0.4	0.08	10	2	0.4	0.08	10	2	0.4	0.08
1) TF 57-19 (Wildtype)	12/10	13/12	13/12	13/11	52/59	52/53	45/47	27/30	61/66	65/66	54/53	28/23
2) MTF 121 (Mutant 1)	80/75	69/69	64/65	53/44	59/53	54/52	48/41	28/20	71/68	71/69	55/52	26/23
3) MTF 123 (Mutant 2)	17/16	15/17	16/16	16/17	56/58	55/60	50/55	36/36	67/73	66/70	57/63	31/35
4) PBS/BSA	15/16	15/16	15/18	15/19	15/16	17/17	18/18	15/18	15/17	15/16	15/15	15/16

APPENDIX 1 - Sheet (a)

```
HSIGCC4   2009 bases
Human ig germline g-e-a region a: gamma-1 constant region

ID    HSIGCC4    standard; DNA; PRI; 2009 BP.

AC    J00228;

DT    23-APR-1990 (reference update)
DT    18-JUL-1985 (incorporated)

DE    Human ig germline g-e-a region a: gamma-1 constant
DE    region

KW    constant region; gamma-immunoglobulin; germ line;
KW    hinge exon; immunoglobulin; immunoglobulin heavy
KW    chain.

OS    Homo sapiens (human)
OC    Eukaryota; Metazoa; Chordata; Vertebrata; Tetrapoda;
OC    Mammalia; Eutheria; Primates.

RN    [1] (bases 1-2009)
RA    Ellison J.W., Berson B.J., Hood L.E.;
RT    "The nucleotide sequence of a human immunoglobulin
RT    c-gamma-1 gene";
RL    Nucleic Acids Res. 10:4071-4079(1982).

RN    [2] (bases 469-1070, 1465-1821)
RA    Takahashi N., Ueda S., Obata M., Nikaido T.,
RA    Nakai S., Honjo T.;
RT    "Structure of human immunoglobulin gamma genes:
RT    Implications for evolution of a gene family";
RL    Cell 29:671-679(1982).

CC    [1] and [2] report that nucleotide divergence among
CC    the four gamma genes is much greater in the hinge
CC    regions than anywhere else.  [2] also reports the
CC    hinge regions of gamma-2, gamma-3, gamma-4, a gamma
CC    pseudogene, and the 5' flanking, ch2, and ch3
CC    domains of the gamma genes.
CC
CC    this entry is part of a multigene region (region a)
CC    containing the gamma-3, gamma-1, pseudo-epsilon, and
CC    alpha-1 genes.  see segment 1 for more comments.

      Key              Location/Qualifiers

FT    CDS              210..503
FT                     /note="Ig gamma-1 heavy chain
```

APPENDIX 1 - cont. Sheet (b)

```
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FT   conflict           563..563
FT                      /citation=([1],[2])
FT                      /note="T in [1]; c in [2]"
FT   conflict           593..593
FT                      /citation=([1],[2])
FT                      /note="C in [1]; t in [2]"
FT   conflict           614..614
FT                      /citation=([1],[2])
FT                      /note="G in [1]; a in [2]"
FT   conflict           633..633
FT                      /citation=([1],[2])
FT                      /note="G in [1]; gg in [2]"
FT   conflict           643..643
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FT                      /note="G in [1]; a in [2]"
FT   conflict           654..654
FT                      /citation=([1],[2])
FT                      /note="G in [1]; a in [2]"
FT   conflict           684..684
FT                      /citation=([1],[2])
FT                      /note="C in [1]; cc in [2]"
FT   conflict           692..692
FT                      /citation=([1],[2])
FT                      /note="G in [1]; a in [2]"
FT   conflict           765..766
FT                      /citation=([1],[2])
FT                      /note="Aa in [1]; a in [2]"
FT   CDS                892..936
FT                      /note="Ig gamma-1 heavy chain
FT                      c-region hinge"
FT   CDS                1055..1384
FT                      /note="Ig gamma-1 heavy chain
FT                      c-region ch2 domain"
FT   conflict           1475..1475
FT                      /citation=([1],[2])
FT                      /note="C in [1]; cc in [2]"
FT   CDS                1481..1803
FT                      /note="Ig gamma-1 heavy chain
FT                      c-region ch3 domain"
FT   conflict           1578..1578
FT                      /citation=([1],[2])
FT                      /note="T in [1]; c in [2]"

SQ   Sequence 2009 BP; 418 A; 698 C; 566 G; 327 T; 0
SQ   Other;
```

APPENDIX 1 - cont. Sheet (c)

```
          10         20         30         40         50         60
   1 AGCTTTCTGG GGCAGGCCAG GCCTGACCTT GGCTTTGGGG CAGGGAGGGG GCTAAGGTGA
  61 GGCAGGTGGC GCCAGCAGGT GCACACCCAA TGCCCATGAG CCCAGACACT GGACGCTGAA
 121 CCTCGCGGAC AGTTAAGAAC CCAGGGGCCT CTGCGCCTGG GCCCAGCTCT GTCCCACACC
 181 GCGGTCACAT GGCACCACCT CTCTTGCAGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT
 241 GGCACCCTCC TCCAAGAGCA CCTCTGGGGG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA

         310        320        330        340        350        360
 301 CTACTTCCCC GAACCGGTGA CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTGCA
 361 CACCTTCCCG GCTGTCCTAC AGTCCTCAGG ACTCTACTCC CTCAGCAGCG TGGTGACCGT
 421 GCCCTCCAGC AGCTTGGGCA CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA
 481 CACCAAGGTG GACAAGAAAG TTGGTGAGAG GCCAGCACAG GGAGGGAGGG TGTCTGCTGG
 541 AAGCAGGCTC AGCGCTCCTG CCTGGACGCA TCCCGGCTAT GCAGCCCCAG TCCAGGGCAG

         610        620        630        640        650        660
 601 CAAGGCAGGC CCCGTCTGCC TCTTCACCCG GAGCCTCTGC CCGCCCCACT CATGCTCAGG
 661 GAGAGGGTCT TCTGGCTTTT TCCCAGGCTC TGGGCAGGCA CAGGCTAGGT GCCCCTAACC
 721 CAGGCCCTGC ACACAAAGGG GCAGGTGCTG GGCTCAGACC TGCCAAGAGC CATATCCGGG
 781 AGGACCCTGC CCCTGACCTA AGCCCACCCC AAAGGCCAAA CTCTCCACTC CCTCAGCTCG
 841 GACACCTTCT CTCCTCCCAG ATTCCAGTAA CTCCCAATCT TCTCTCTGCA GAGCCCAAAT

         910        920        930        940        950        960
 901 CTTGTGACAA AACTCACACA TGCCCACCGT GCCCAGGTAA GCCAGCCCAG GCCTCGCCCT
 961 CCAGCTCAAG GCGGGACAGG TGCCCTAGAG TAGCCTGCAT CCAGGGACAG GCCCCAGCCG
1021 GGTGCTGACA CGTCCACCTC CATCTCTTCC TCAGCACCTG AACTCCTGGG GGGACCGTCA
1081 GTCTTCCTCT TCCCCCCAAA ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC
1141 ACATGCGTGG TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
```

APPENDIX 1 - cont. Sheet (d)

```
               1210       1220       1230       1240       1250       1260
1201 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA CAACAGCACG
1261 TACCGGGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT GGCTGAATGG CAAGGAGTAC
1321 AAGTGCAAGG TCTCCAACAA AGCCCTCCCA GCCCCCATCG AGAAAACCAT CTCCAAAGCC
1381 AAAGGTGGGA CCCGTGGGGT GCGAGGGCCA CATGGACAGA GGCCGGCTCG GCCCACCCTC
1441 TGCCCTGAGA GTGACCGCTG TACCAACCTC TGTCCTACAG GGCAGCCCCG AGAACCACAG

               1510       1520       1530       1540       1550       1560
1501 GTGTACACCC TGCCCCCATC CCGGGATGAG CTGACCAAGA ACCAGGTCAG CCTGACCTGC
1561 CTGGTCAAAG GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
1621 GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT CTTCCTCTAC
1681 AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA ACGTCTTCTC ATGCTCCGTG
1741 ATGCATGAGG CTCTGCACAA CCACTACACG CAGAAGAGCC TCTCCCTGTC TCCGGGTAAA

               1810       1820       1830       1840       1850       1860
1801 TGAGTGCGAC GGCCGGCAAG CCCCGCTCCC CGGGCTCTCG CGGTCGCACG AGGATGCTTG
1861 GCACGTACCC CCTGTACATA CTTCCCGGGC GCCCAGCATG GAAATAAAGC ACCCAGCGCT
1921 GCCCTGGGCC CCTGCGAGAC TGTGATGGTT CTTTCCACGG GTCAGGCCGA GTCTGAGGCC
1981 TGAGTGGCAT GAGGGAGGCA GAGCGGGTC
```

CLAIMS

1. A molecule which comprises an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain which constant regions are of a particular isotype and have one or more allotypic determinants

wherein said amino acid sequence is substantially homologous to the amino acid sequence of said constant region, but has been altered so that it is without at least one of said allotypic determinants, by making the amino acid residues at the site of an allotypic determinant identical to the amino acid residues from the corresponding position in another equivalent immunoglobulin constant region of a different isotype.

2. A molecule according to claim 1 which comprises an amino acid sequence derivable from part or all of a human immunoglobulin constant region.

3. A molecule according to claim 1 or claim 2 which comprises one or more polypeptides together with said amino acid sequence.

4. A molecule according to claim 3 wherein the polypeptide comprises a functional biological domain.

5. A molecule according to claim 4 wherein the functional biological domain comprises a binding domain.

6. A molecule according to claim 5 wherein the binding domain is an immunoglobulin-like binding domain.

7. A molecule according to claim 6 in which said immunoglobulin-like binding domain and said amino acid sequence are derivable from the same or different sources.

8. A molecule according to any one of claims 1 to 7 wherein the constant region is from an immunoglobulin of the isotype IgG.

9. A molecule according to claim 8 wherein the isotype subclass is IgG1 and the molecule no longer has one or more of the allotypic determinants 1,2,3 and 17.

10. A molecule according to claim 8 wherein the isotype subclass is IgG2 and the molecule no longer has the allotypic determinant 23.

11. A molecule according to claim 8 wherein the isotype subclass is IgG3 and the molecule no longer has one or

more of the allotypic determinants 11,5,13,14,10,6,24,21, 15,16,26 and 27.

12. A molecule according to any one of claims 1 to 7 wherein the constant region is from an immunoglobulin of the isotype IgA2 and the molecule no longer has either or both of the allotypic determinants 1 and 2.

13. A pharmaceutical preparation which comprises a molecule according to any one of claims 1 to 12.

14. A reagent which comprises a molecule according to any one of claims 1 to 12.

15. A nucleotide sequence encoding a molecule according to any one of claims 1 to 12.

16. A cloning or expression vector comprising a nucleotide sequence according to claim 15.

17. A host cell comprising a cloning or expression vector according to claim 16.

18. A method of preparing a molecule according to any one of claims 1 to 12 which comprises the steps of:
(a) identifying a constant region of an immunoglobulin

heavy chain;

(b) comparing the identified constant region with constant regions from immunoglobulin heavy chains of the same isotype to locate allotypic determinants in the identified constant region;

(c) obtaining the coding sequence for the identified constant region having allotypic determinants;

(d) altering the coding sequence so that it codes for a molecule without at least one of said allotypic determinants and by making the amino acid residues at the site for an allotypic determinant identical to the amino acid residues from the corresponding position in an equivalent immunoglobulin constant region of an isotype different to that of said identified constant region;

(e) using said altered coding sequence in an expression system to produce a said molecule.

19. A method of treating a patient which comprises administering a pharmaceutical preparation according to claim 13.

Fig. 1.

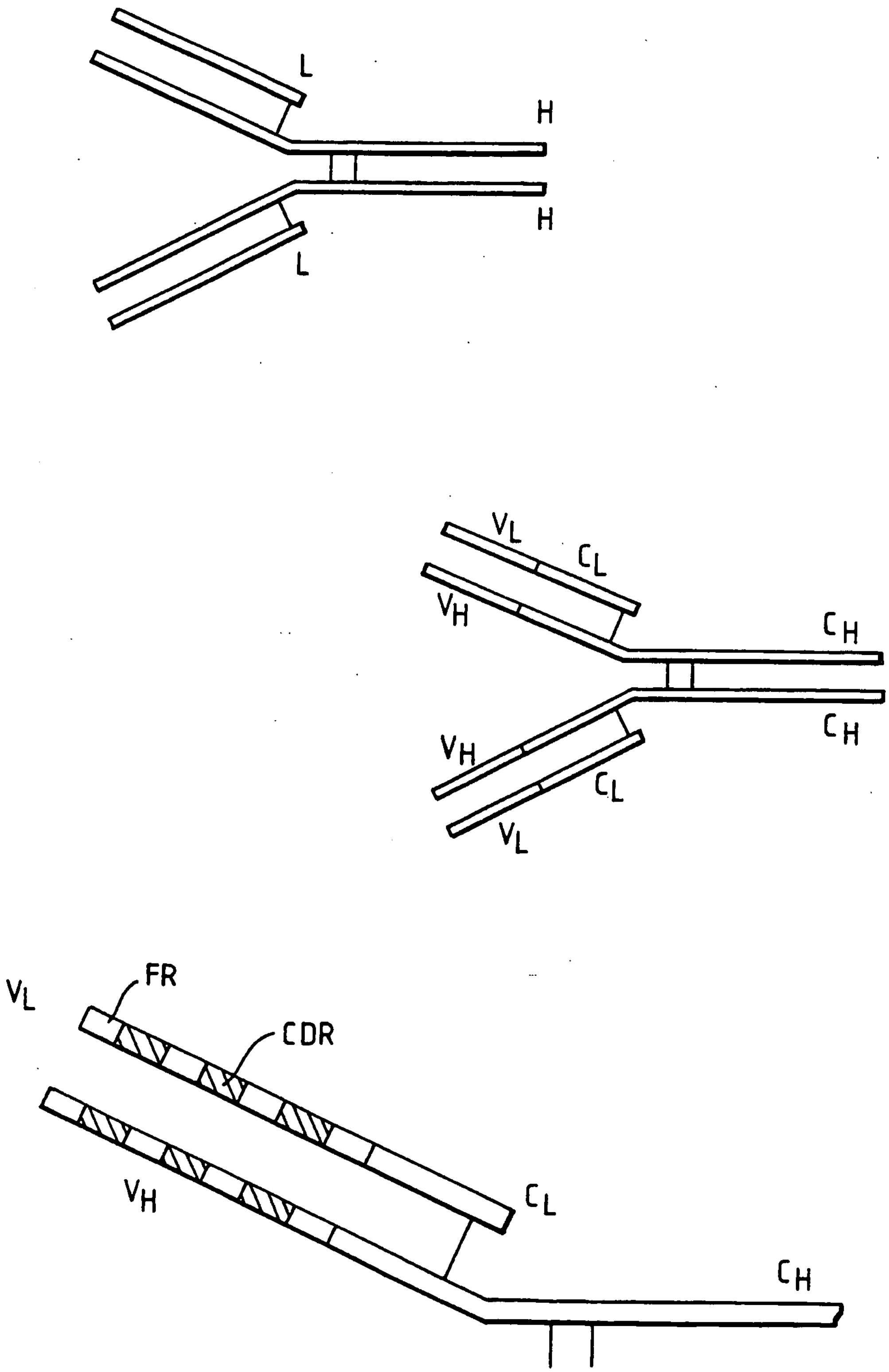
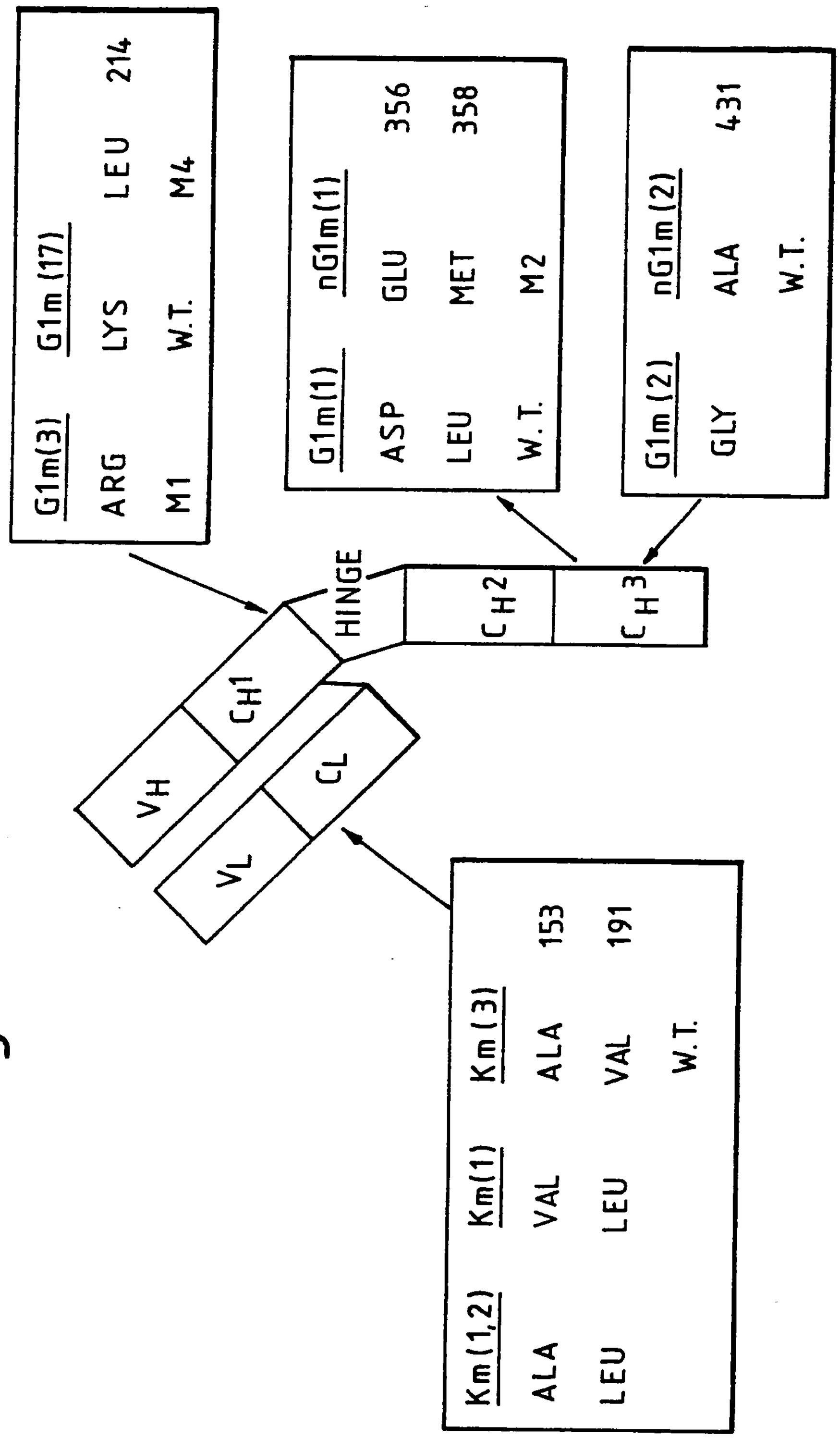


Fig. 2.


G1m(3) G1m (17)
ARG LYS LEU 214
M1 W.T. M4
G1m(1) nG1m(1)
ASP GLU 356
LEU MET 358
W.T. M2
G1m (2) nG1m (2)
GLY ALA 431
W.T.
HINGE
C_H1
C_H2
C_H3
VH
VL
CL
Km (1,2) Km(1) Km (3)
ALA VAL ALA 153
LEU LEU VAL 191
W.T.

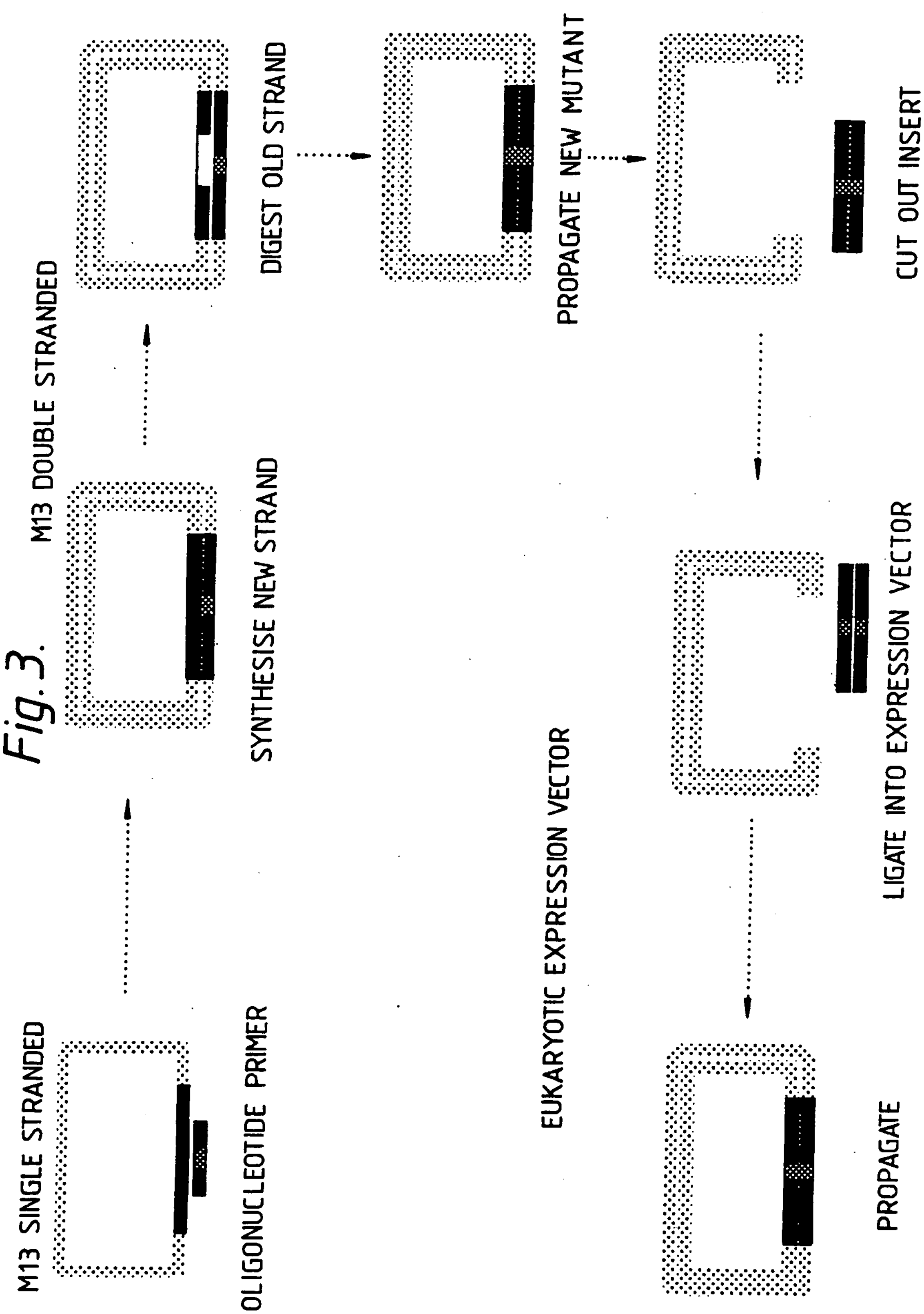

M13 SINGLE STRANDED
OLIGONUCLEOTIDE PRIMER
SYNTHESISE NEW STRAND
M13 DOUBLE STRANDED
DIGEST OLD STRAND
PROPAGATE NEW MUTANT
CUT OUT INSERT
EUKARYOTIC EXPRESSION VECTOR
LIGATE INTO EXPRESSION VECTOR
PROPAGATE

Fig. 3.

Fig. 4a.

<u>Human immunoglobulin sequences CH1 region</u>

```
AlaSerThrLysGlyProSerValPheProLeuAlaProSerSerLysSerThrSerGly  IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  - Arg -  -  -  Glx  IgG2
 -  -  -  -  -  -  -  -  -  -  -  -  - Cys - Arg -  -  -  -    IgG3
 -  -  -  -  -  -  -  -  -  -  -  -  - Cys - Arg -  -  -  Glu  IgG4

GlyThrAlaAlaLeuGlyCysLeuValLysAspTyrPheProGluProValThrValSer  IgG1
Ser -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG2
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG3
Ser -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG4

TrpAsnSerGlyAlaLeuThrSerGlyValHisThrPheProAlaValLeuGlnSerSer  IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG2
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG3
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG4

GlyLeuTyrSerLeuSerSerValValThrValProSerSerSerLeuGlyThrGlnThr  IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  -  - AsnPhe -  -  -  -    IgG2
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -    IgG3
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  - Lys -    IgG4

                                                Glm(17)
TyrIleCysAsnValAsnHisLysProSerAsnThrLysValAspLysLysVal        IgG1
 - Thr -  -  - Asp -  -  -  -  -  -  -  -  -  - Thr -         IgG2
 - Thr -  -  -  -  -  -  -  -  -  -  -  -  -  - Arg -         IgG3
 - Thr -  -  - Asp -  -  -  -  -  -  -  -  -  - Arg -         IgG4
```

Fig. 4b.

Human immunoglobulin sequences hinge region

GluProLys	SerCysAspLysThrHisThrCysProPro	IgG1
GlxArgLys	CysCys Val Glx CysProPro	IgG2
GluLeuLysThrProLeuGlyAspThrThrHisThrCysProArgCysProGlu		IgG3
GluSerLysTyrGly	ProProCysProPro	IgG4

	IgG1
	IgG2
ProLysSerCysAspThrProProProCysProArgCysProGluProLysSer	IgG3
	IgG4

	IgG1
	IgG2
CysAspThrProProProCysProArgCysProGluProLysSerCysAspThr	IgG3
	IgG4

CysPro	IgG1
CysPro	IgG2
ProProProCysProArgCysPro	IgG3
CysPro	IgG4

Fig. 4c.

Human immunoglobulin sequences CH2 region

Ala	Pro	Glu	Leu	Leu	Gly	Gly	IgG1
-	-	Pro	Val	Ala		-	IgG2
-	-	-	-	-	-	-	IgG3
-	-	-	Phe	-	-	-	IgG4

Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG4

Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Gln	-	-	-	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Gln	-	Lys	-	IgG3
-	-	-	-	-	-	-	-	-	-	Gln	-	-	-	-	-	Gln	-	-	-	IgG4

Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Phe	-	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Phe	-	IgG4

Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	IgG1
-	-	Phe	-	-	-	-	-	-	-	-	Val	-	-	-	-	-	-	-	-	IgG2
-	-	Phe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG4

Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	IgG1
-	-	-	-	-	-	-	-	-	Gly	-	-	-	-	-	-	-	-	-	-	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	Gly	-	-	Ser	Ser	-	-	-	-	-	-	IgG4

Lys	Ala	Lys	IgG1
-	Thr	-	IgG2
-	Thr	-	IgG3
-	-	-	IgG4

Fig. 4d.

Human immunoglobulin sequences CH3 region

```
                                                                    G1m(1)
         GlyGlnProArgGluProGlnValTyrThrLeuProProSerArgAspGlu    IgG1
          -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  - Glu -    IgG2
          -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  - Glu -    IgG3
          -  -  -  -  -  -  -  -  -  -  -  -  -  -  - GlnGlu -    IgG4

G1m(1)
LeuThrLysAsnGlnValSerLeuThrCysLeuValLysGlyPheTyrProSerAspIle    IgG1
Met -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -      IgG2
Met -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -      IgG3
Met -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -      IgG4

AlaValGluTrpGluSerAsnGlyGlnProGluAsnAsnTyrLysThrThrProProVal    IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  - Met    IgG2
 -  -  -  -  - Ser -  -  -  -  -  - Asn -  -  -  -  -  - Met    IgG3
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -        IgG4

LeuAspSerAspGlySerPhePheLeuTyrSerLysLeuThrValAspLysSerArgTrp    IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -     IgG2
 -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -     IgG3
 -  -  -  -  -  -  -  -  -  -  - Arg -  -  -  -  -  -  -  -     IgG4

                                             G1m(2)
GlnGlnGlyAsnValPheSerCysSerValMetHisGluGlyLeuHisAsnHisTyrThr    IgG1
 -  -  -  -  -  -  -  -  -  -  -  -  - Ala -  -  -  -  -  -     IgG2
 -  -  -  - Ile -  -  -  -  -  -  -  - Ala -  -  - ArgPhe -     IgG3
 -  -  -  -  -  -  -  -  -  -  -  -  - Ala -  -  -  -  -  -     IgG4

GlnLysSerLeuSerLeuSerProGlyLys    IgG1
 -  -  -  -  -  -  -  -  -  -     IgG2
 -  -  -  -  -  -  -  -  -  -     IgG3
 -  -  -  -  -  -  - Leu -  -     IgG4
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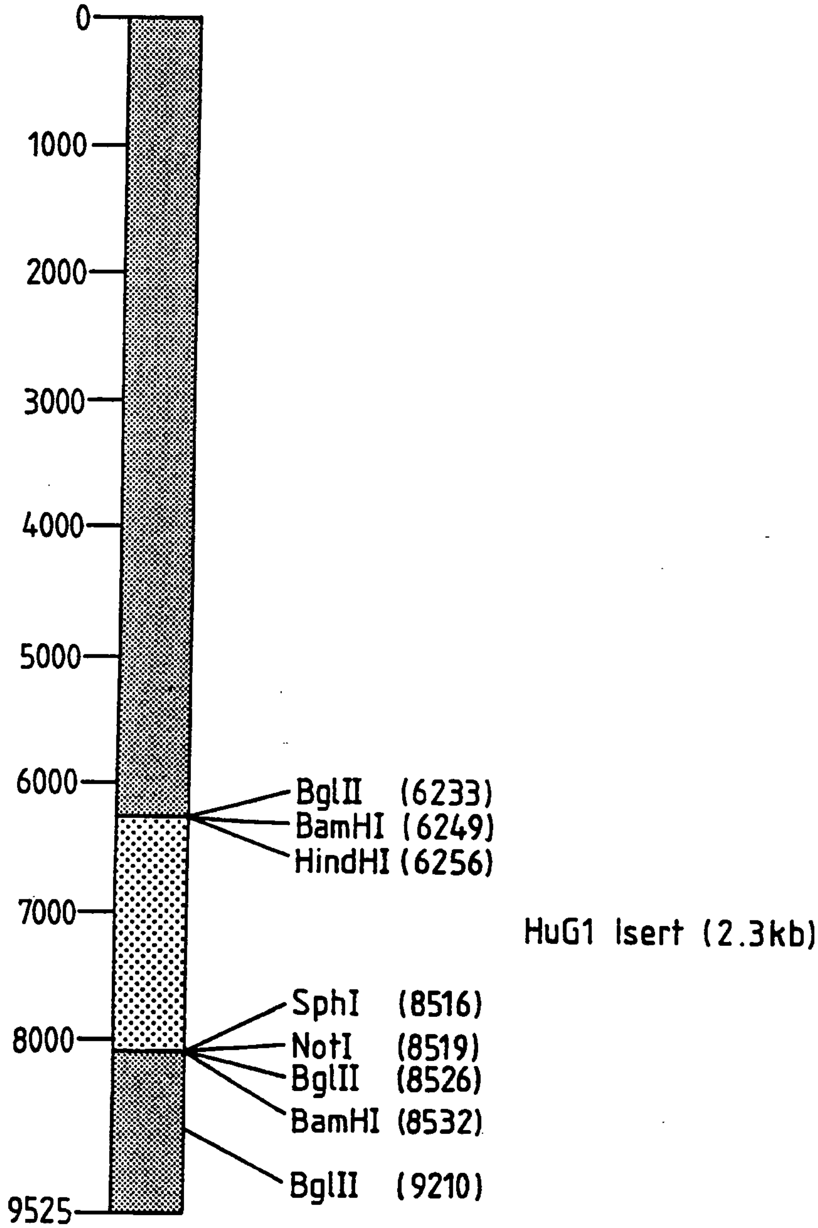
Fig. 5.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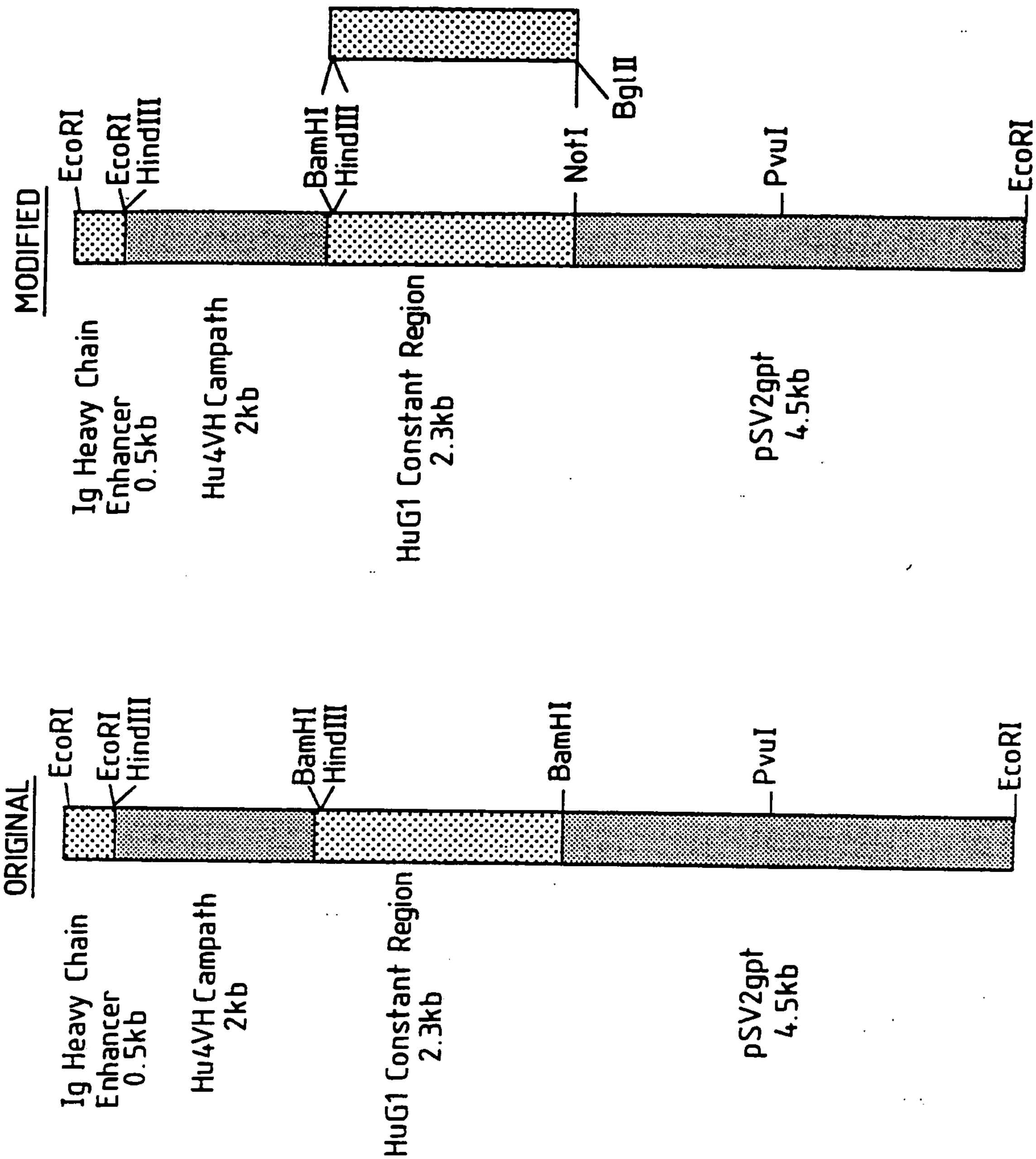
BglII (9210)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HuG1 Constant Region 2.3kb
pSV2gpt 4.5kb
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BamHI
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PvuI
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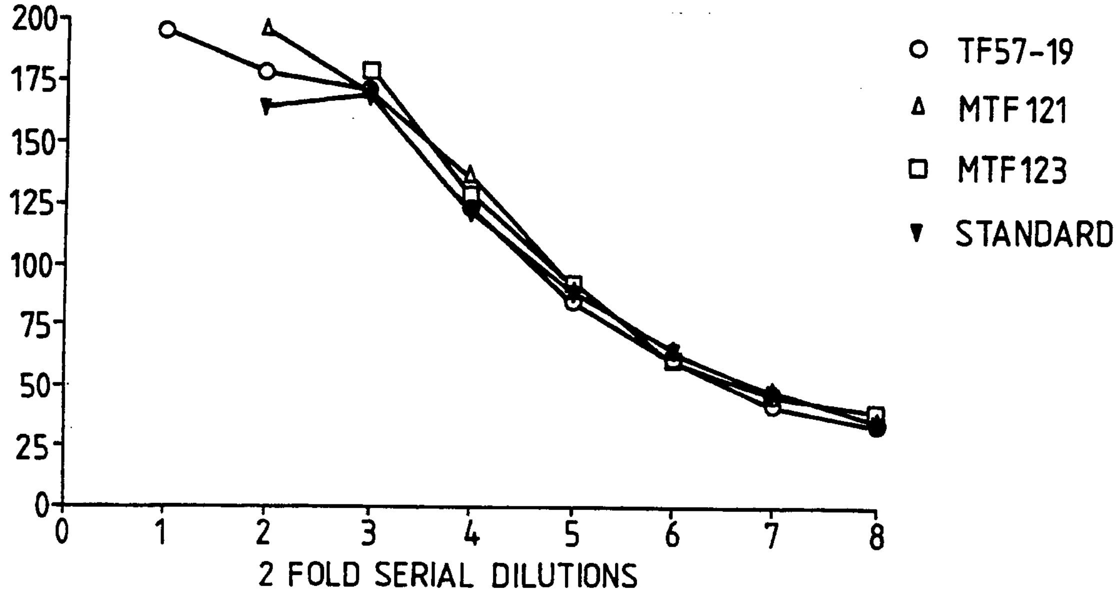
Hu4VH Campath 2kb
HuG1 Constant Region 2.3kb
pSV2gpt 4.5kb

Fig. 6.

Fig. 7.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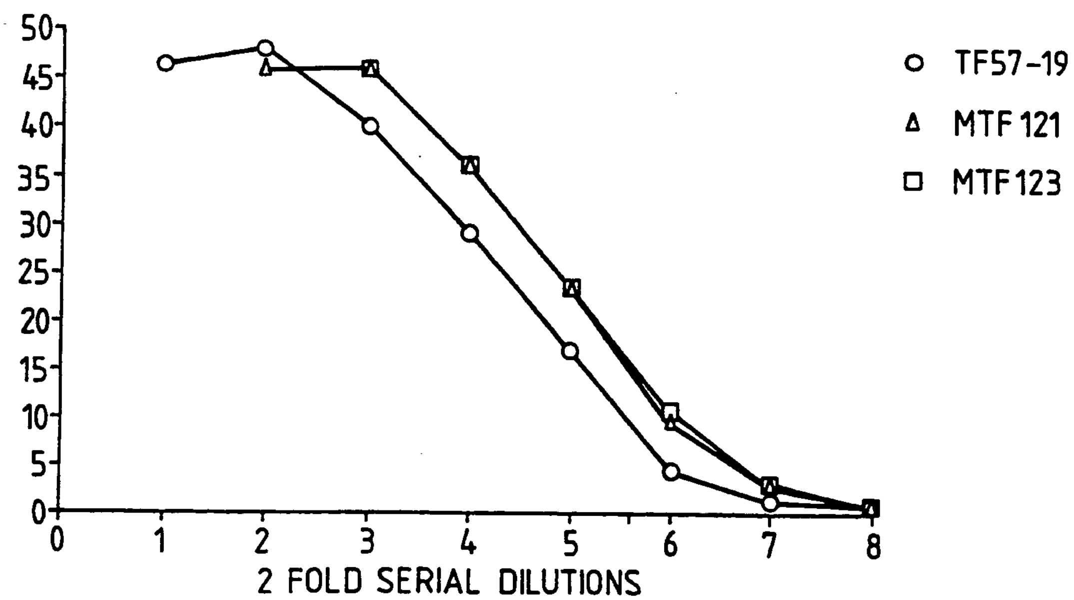
MTF 121
MTF 123
STANDARD

Fig. 8.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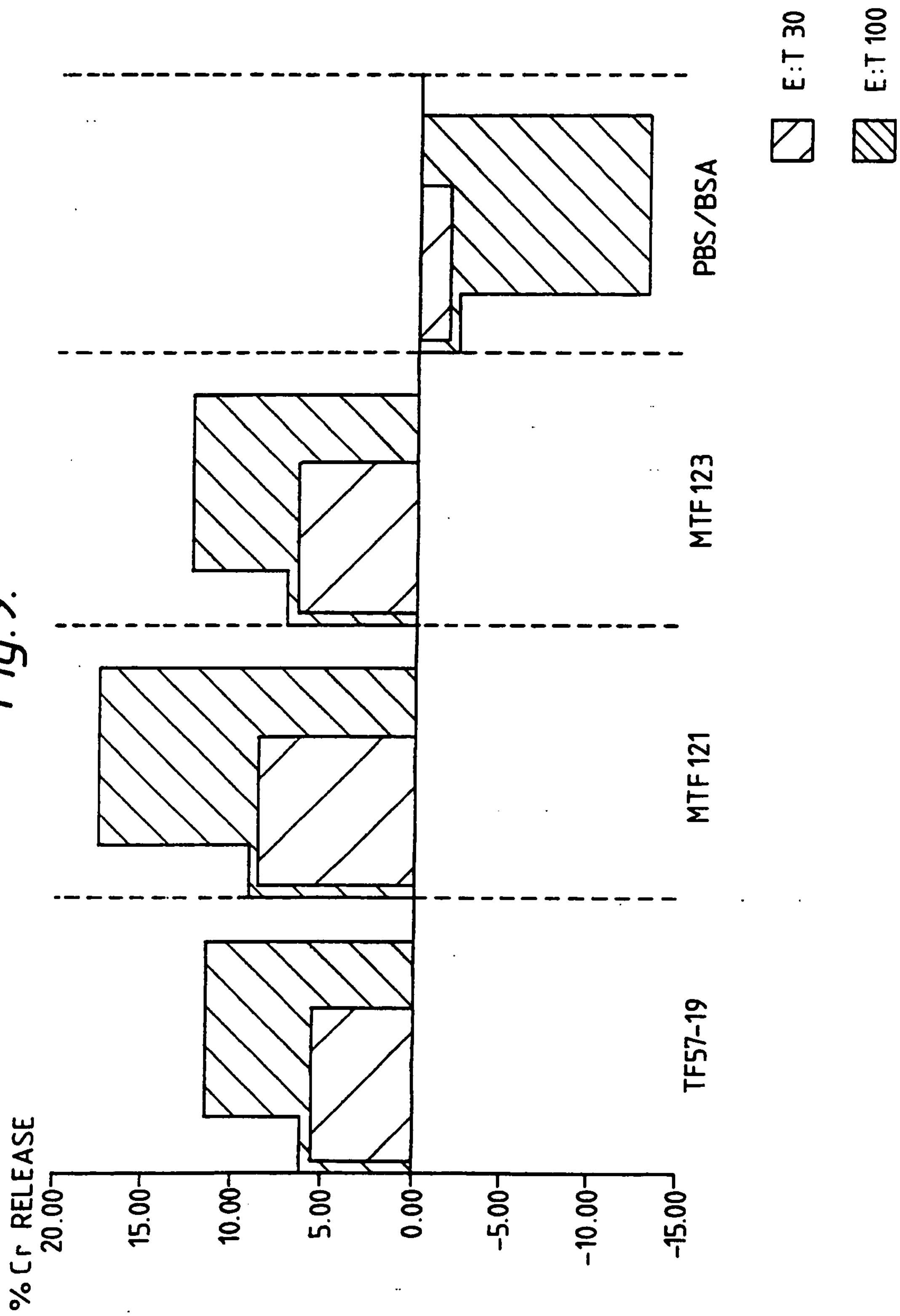
MTF 121
MTF 123

Fig. 9.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PBS/BSA
E:T 30
E:T 100

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00445

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 K 15/28 C 12 P 21/08 C 12 N 5/10
C 12 N 15/13 A 61 K 39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	The Journal of Immunology, vol. 143, no. 11, 1 December 1989, (Baltimore, MD, US), G. HEINRICH et al.: "Characterization of a human T cell-specific antibody (CD7) with human constant and mouse variable regions", pages 3589-3597, see the whole document, especially front page abstract; page 3591, left-hand column: "Cloning of human IgG1 genes"; page 3591, right-hand column, lines 9-13; page 3592, right-hand column, lines 13-19; page 3593, line 34 - page 3594, line 4 ---	1-19
A	NATURE, vol. 333, 30 June 1988, (London, GB), R.G. MAGE: "Designing antibodies for human therapies", pages 807-808, see the article (cited in the application) ---	1-19

-/-

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04-06-1992	30. 06. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nicole De Ble

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,0328404 (MEDICAL RESEARCH COUNCIL) 16 August 1989, see the whole document ---	1-19
A	W.E. PAUL, M.D.: "Fundamental Immunology", 1984, Raven Press, New York, US; chapter 9: J.B. FLEICHMAN et al.: "Immunoglobulins: Allotypes and Idotypes", see the whole document -----	1-19

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 19 is directed to a method of the human body the search has been carried out and based on the alleged effects of the compositon.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200445 SA 57491

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 23/06/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0328404	16-08-89	AU-A- 3062689	06-09-89
		WO-A- 8907452	24-08-89
		GB-A- 2216126	04-10-89

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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